



Proximate Composition and Amino Acid Profile of Tigernut Wastes for Bioethanol Production

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Abstract

Agricultural wastes are rich in nutrients. Proximate composition of tigernut (*Cyperus esculentus*) waste was determined for application as a substrate for bioethanol production. Pre-treatment was done prior to the proximate and amino acid analysis. The waste sample coded as TNW (Tigernut waste) was investigated using the Applied Biosystems PTH Amino Acid Analyzer) demonstrated intrinsic proximate composition which may encourage bioethanol yield. The percentage compositions are; Crude protein (10.15), Fat (4.035), Ash content (2.68), Crude fibre (23), Moisture (5.38) and Nitrogen free extract (NFE) (54.75). The percentage ethanol yield for all the samples fell within the range of 46 - 64% at 360⁰C and 43 - 58% at 420⁰C. Therefore, high carbohydrate yields and other inherent compositions recommends it as precursors for activated biomass in bioethanol production. Amino acid profile shows that the highest occurring amino acid in tiger nut waste was glutamic acid, while the least occurring is tryptophan.

Keywords: Agricultural waste, amino acid profile, proximate analysis

Introduction

Agricultural wastes or biomass sources other than those used as food are explored to reduce the dependence on food and feeds as substrates for bioethanol production. These wastes include animal wastes, woods, herbaceous plants, crops and forest residues. In Nigeria, large quantities of these wastes are produced annually and are vastly under-utilised (Obi *et al.* 2016). Since agricultural wastes are readily available at little

or no costs, they have the potential to provide low cost adsorbent for cleaning our environment. They can also be converted to wealth in large scale application using simple technology (Awogbemi, 2021b). Since the costs of fossils fuels are continuously rising and their availability highly reduced, there is need to accurately analyse the compositions of local agricultural wastes for use in bioethanol production.

This paper investigated the proximate composition of an agricultural waste *Cyperus esculentus* (Tigernut) and its proximate and amino acid profile for the production of bioethanol.

Methodology

Collection of Agricultural Waste Samples

Comosite dried chaff of tigernut wastes were collected from various markets in Owerri Municipal Council, Imo State, Nigeria. The dried samples of tigernut waste were futher sun dried for 48 h and sorted to remove sand and other debris. The chaff was ground using a milling machine at the Milling laboratory of Food Science Technology Department, Federal University of Technology, Owerri, Nigeria.

Proximate Analyses of Samples

Analysis of moisture content of waste samples

The method of AOAC (2019) was adopted with slight modifications. The water content was determined by weighing out 2 g into glass Petri dish, which has been previously dried and weighed. The dish, containing the samples, was placed inside hot air oven and allowed for 5 h at $130\pm 3^{\circ}\text{C}$, to dry to constant weight. It was then removed and allowed to cool for ten minutes in a desiccator, before weighing. Moisture content of the samples was computed using equation:

% Moisture content =

$$\frac{\text{Weight loss on drying, g}}{\text{Weight test portion, g}} \times 100$$

Nitrogen determination by micro Kjeldahl method (crude protein)

This was carried out as described by AOAC (2019). The nitrogen component of protein and other compounds was converted to ammonium sulphate, by acid digestion, with boiling sulphuric acid. Five millilitre (5 mL) of sample was placed in Kjeldahl flask, and about 200 mg of catalyst mixture (potassium sulphate, copper sulphate and

selenium powder) was added. Then 10 mL of concentrated sulphuric acid was added to the content of the flask. It was gently heated for few minutes, until frothing ceased. Temperature was increased to digest it for 1 hour. Afterward, it was allowed to cool, and then made up 100 mL with distilled water. 5 mL of digested solution was pipetted into the distillation chamber of micro-Kjeldhal distillation apparatus and 10 mL of 40% sodium hydroxide solution was added. It was distilled into 10 mL of 4% boric acid, containing mixed indicator (colour from red-green was noted), and titrated with standard 0.01N or 0.02 N hydrochloric acid to grey end point.

$$\% \text{ N} = \frac{(a-b) \times 0.01 \times 14.0057 \times c \times 100}{d \times e}$$

a= titre value for the sample

b= titre value for the blank

c= Volume to which digest is made up with distilled water

d= Aliquot taken for distillation

e= Weight of dried sample (mg)

To percentage crude protein, then multiplied by necessary conversion factor (6.25).

Ash Determination

The method of AOAC (2019) was adopted. Two grammes (2 g) test portion was weighed into porcelain crucible and placed in muffle furnace. It was then pre-heated to 600°C , and held at this temperature for 2 h. Crucible was directly transferred to desiccator, cooled, and weighed immediately, percent ash was reported to two decimal places % (w/w).

Fat Determination (ether-extract)

The method of AOAC (2019), with slight modifications was adopted. A soxhlet extraction apparatus and 250 ml quickfit flask which has been previously dried in the oven was fitted up. Two grammes (2 g) of sample was weighed and transferred to a fat free extraction thimble, plugged lightly with cotton wool. The thimble was placed in the extractor and about 150 cm^3 of petroleum

ether (B.P. 40-60°C) was added into the flask, until it siphons over once. The source of heat (electrothermal heating mantle) was adjusted so that the ether boils gently and left to siphon over for at least 6 h. The flask (which now contains all the oil) was detached. Extract (oil) was filtered through Whatman filter paper into weighed beaker, washing paper finally with small portion of hot fresh ether. Solvent Evaporated at 100 degree centigrade and dry beaker containing residue in an air oven 1 hour at 100-105 degree centigrade. Reported as % oil to second decimal place.

Crude Fibre Determination

The method of AOAC (2019), was adopted slight modification. Defatted ground sample was

transferred from fat determination into 250 ml quickfit flask, 150 ml of 1.25% sulphuric acid was added and fitted to reflux condenser. Reflux for 30 minutes, cool and filter using Buchner funnel fitted with Whatman filter paper. Rinsed three times with hot distilled water, dried and transferred the residue into quickfit flask. 150 ml of 1.25% Sodium hydroxide and reflux was added for 30 min. Filter using Buchner funnel, and rinsed three times with hot distilled water, once with 1.25% sulphuric acid and finally with 95% ethanol. The filter paper containing residue was removed into porcelain crucible and dried in oven 2h at 130°C. Cooled in dessicator, ash at 550⁰ ± 10⁰C in murfle furnance, cooled in dessicator and weighed. Crude fibre, %. materials, provides the estimate of the ash.

Results and Discussion

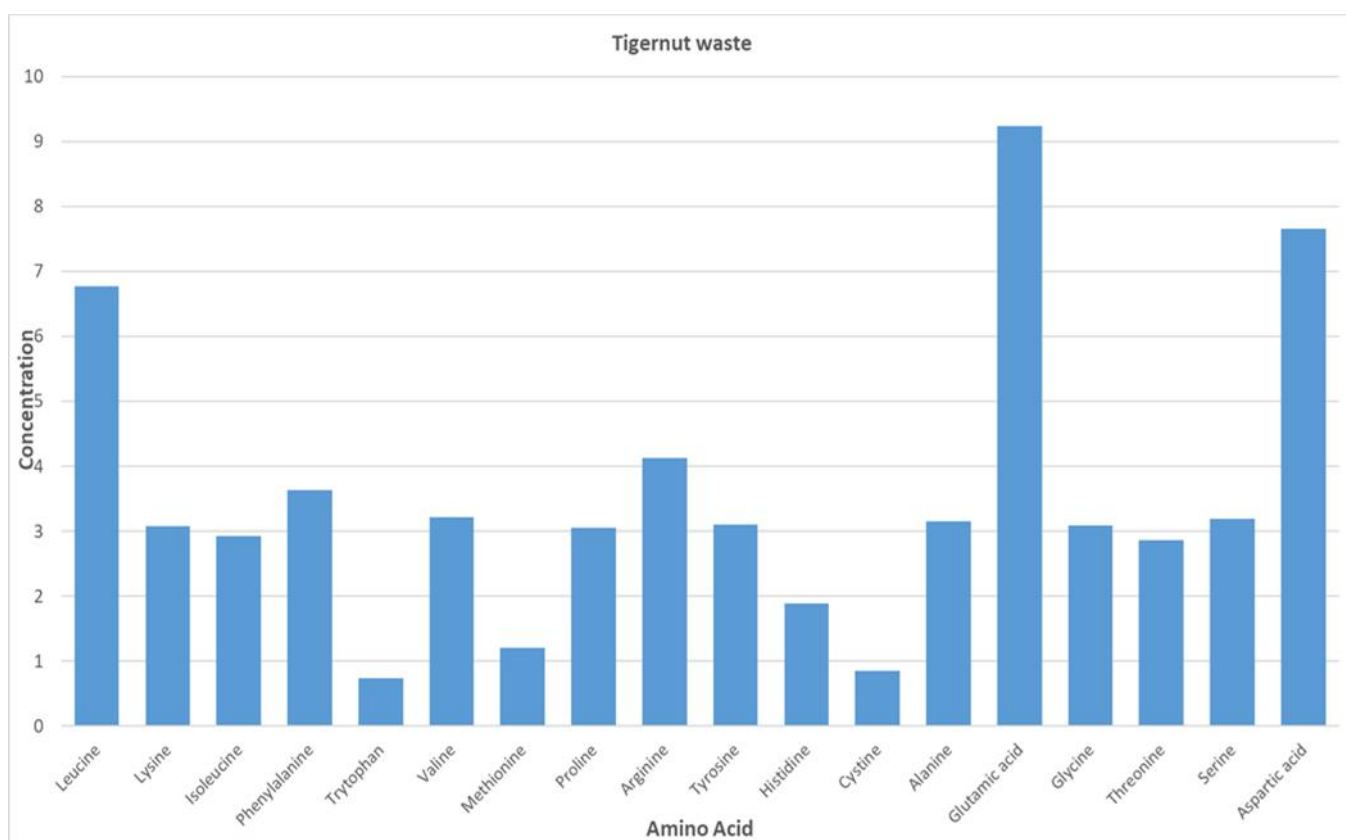


Fig 1. Amino acid composition of Tigernut waste

Figure 1 shows that the highest occurring amino acid in tiger nut waste is glutamic acid, while the least occurring is tryptophan. TNW contains roughly the same quantity of lysine, proline, tyrosine, glycine, alanine, and serine. The presence of glutamic acid as the highest occurring amino acid in TNW is noteworthy because glutamic acid can serve as a valuable nutrient source for these microorganisms, potentially enhancing their fermentation efficiency (Yu et al., 2022). The low amount of tryptophan in TNW is not necessarily negative for bioethanol production, as tryptophan is not typically a critical nutrient for ethanol-producing microorganisms (NBI, 2024).

The fact that TNW contains roughly the same quantity of lysine, proline, tyrosine, glycine,

alanine, and serine is important because these amino acids can collectively contribute to the overall nutrient content available to ferment microorganisms (Singh, 2018). A balanced supply of these amino acids is advantageous for supporting microbial growth and the fermentation process.

In bioethanol production, the efficiency of fermentation is a crucial factor. The nutrient content in the feedstock, including amino acids, can influence the growth and metabolic activity of the ethanol-producing microorganisms (Arijana et al., 2023). The balanced amino acid composition in TNW suggests that it could be a suitable substrate for fermentation, potentially leading to more efficient and productive bioethanol production.

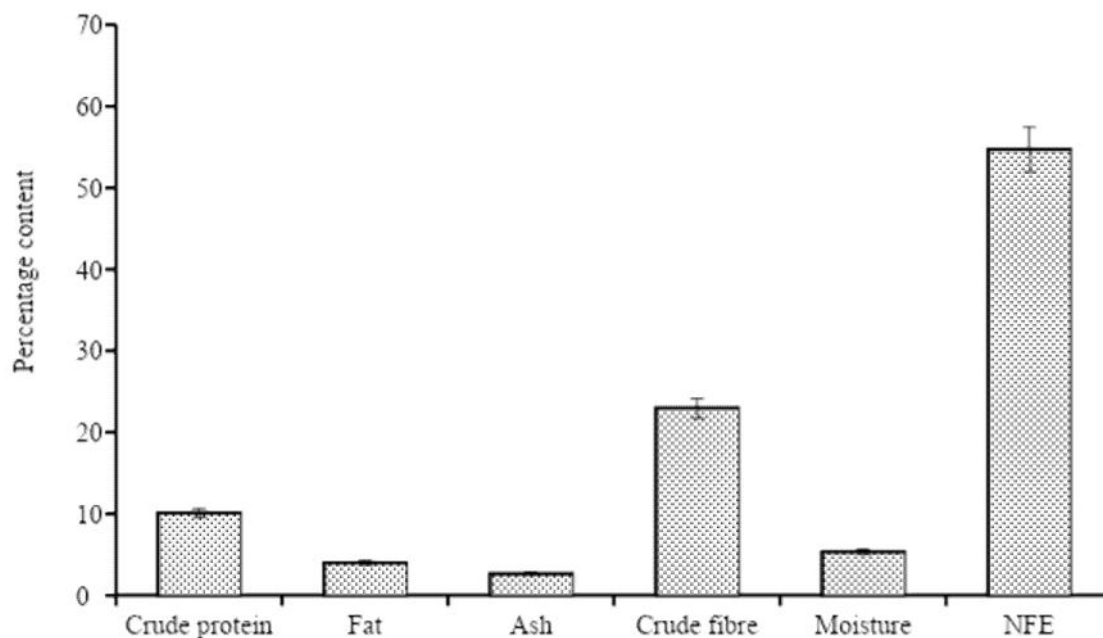


Figure 2: Proximate composition of tignut waste

Bioethanol production typically requires carbohydrates for fermentation (Peters, 2006), and the protein content in the samples is relatively low. Excessive protein can interfere with the fermentation process, so the level (10.15%) is within the desired range (Rinastiti et al., 2022). TNW has a slightly higher fat content (4.035%), but it is still within an acceptable range especially

as fat can potentially inhibit fermentation (Ibeogu, I. H. and Eze, J. 2022). Lower ash content in TNW (2.68%) is more favourable for bioethanol production (Adedara et al., 2020). (High crude fiber content, such as that in TNW (23%), is advantageous for bioethanol production (Alabi et al., 2022). Fiber serves as a source of fermentable sugars during the process (Falowo et al., 2023).

Furthermore, the high NFE favours bioethanol production (Tripathi et al 2023).

The results of the proximate analysis of tiger nut residue show that the residue contained a high proportion of carbohydrate as Nitrogen free extract (NFE) (54.75 ± 0.3) as shown in Fig 2. This is consistent with the value (58.80 ± 1.6) as reported by Nata'ala, Farouq, Magashi and Liman (2018). Their sample size might explain the high error margin compared to the present study. From the current study, the high carbohydrate level recorded might be attributed to the fact that tigernut is a tuber crop like cassava and potato, which contain a high amount of carbohydrate of about 46.99% (Gambo and Da'u, 2014). The carbohydrate might not be fully extracted during the process of making tigernut milk that gives rise the residue as a by-product. This is in agreement with the findings of Wayah and Shehu (2013) that reported a carbohydrate content of 43.0%, which is lower than the reported 54.75%. This could be attributed to the differences in the processes of extraction in making of tiger nut milk (*kunun aya*).

The crude fat content of the residue was found to be (4.035), which is far lower than 17.50% reported by Nata'ala, Farouq, Magashi and Liman (2018) and 35.43% reported for dried tiger nut (Oladele and Aina, 2007). This might be attributed to the extreme extraction procedure adopted. Other compositions, crude protein (10.15), ash content (2.68), crude fibre (23), moisture (5.38) are consistent. The results report the values as follows: moisture content (%) 7.83 ± 0.58 , ash content (%) 2.33 ± 0.29 , crude fat (%) 17.50 ± 0.50 , crude fiber (%) 10.67 ± 0.29 , crude Protein (%) 2.86 ± 0.13 , and soluble carbohydrate (%) 58.80 ± 1.6 .

Conclusion

Currently, non-fossil energy sources have a very low share of the energy supply in Nigeria. If the agricultural wastes are efficiently harnessed in the production of biobased products such as bioethanol, it will contribute to a large share of

the renewable energy mix as well as reduce the demand for imported fuel sources. Because of their positive environmental impacts, and future economic considerations, numerous institutions are attempting to promote the biobased technology of producing biofuel and biopower. The government of Nigeria should endeavour to develop a biofuel plant for bioethanol alternative fuel for SI engines which can be managed by producing bioethanol from agricultural waste. Based on current research headway, it is clear that bioethanol production from lignocellulosic agricultural residues will undoubtedly become a viable technology to fulfill fuel security soon. Finally, because of the abundance of biomass in Nigeria, sustainable energy will soon overtake the power industry especially through public and private cooperation.

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